

RADIATION CATARACOGENESIS IN RAT LENSES

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THE potential of ionizing radiation, such as x rays, to produce cataracts has been recognized since Chaluppecky's studies in 1897.¹ Since then radiation cataractogenesis has been studied as a function of dose, age and radiation modality.²⁻⁶ The closely reasoned work of a number of investigators has led to the hypothesis that radiation cataracts depend on proliferation of the germinative zone cells of the lens epithelium. These cells are thought to be the progenitor population for aberrantly differentiating cell fibers contributing to the histopathological changes associated with the cataract.⁷⁻¹⁰ The specific mechanism by which the effect is achieved is unknown and continues to be a subject of intense study.

The scanning electron microscope has provided a novel means of examining the cellular relationships in the lens. Such studies have shown that mammalian lens fibers form an intricate cytoarchitecture. Individual fiber cells were shown to have "ball-and-socket" and "tongue-and-groove" attachments on the flattened surfaces of the cortical fibers, in addition to complex interdigitating processes at the terminal ends of fibers along the suture line.¹¹⁻¹⁸

Morphological change in the integrity of the lens is difficult to appreciate from the two-dimensional view provided by light microscopy or trans-

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mission electron microscopy. The aim of the present investigations was to study the morphological development of radiation cataracts over a range of x-ray doses by means of the scanning electron microscope. By observing changes at different times after the initial photonic insult, the progression of the cataract can be ascertained.

MATERIALS AND METHODS

Columbia-Sherman albino rats, four weeks of age, were used in these investigations. The rats were divided into several dose groups in which the right eyes were exposed to 6, 10, 12, 14 or 18 Gy [1 Gray(Gy)=1 joule/kgm=100 rads] of 185 kVp x-irradiation, filtered by 1.0 mm copper, at a dose rate of 2 Gy/min. The contralateral shielded eyes received a calculated dose of no more than 1-2% of the direct radiation and served as a control. The radiation was administered while the animals were anesthetized with sodium pentobarbital.¹⁹ The rats were examined by slit lamp biomicroscopy, and the cataracts were scored as described elsewhere.²⁰⁻²² Forty-six animals exposed to various doses were decapitated on the 45th day post-irradiation. In the time-dependency study, two animals were selected randomly and killed at 12 hours and at one, two, three, four, five, six, eight, 10 and 12 weeks after a dose of 10 Gy x-radiation. Three rats were killed 70 weeks after 10 Gy x-radiation, and one rat, which received only 2 Gy, was killed one year post-irradiation.

Excised eyes were incised to facilitate fixation in phosphate buffered 2.5% glutaraldehyde followed by phosphate buffered 1% OsO₄. Lenses were then dehydrated and critical point dried in Freon 13 before mechanical fracturing.¹⁶ Frequently, spontaneous splitting of the lenses during processing provided convenient fracture planes along the sutures to expose the inner surface. Otherwise intact specimens were split at room temperature by initiating a fracture with a razor blade. The fragments were mounted on specimen holders so that the exposed surfaces faced upward. They were coated with gold and examined with a Jeol JSM-25 SEM.

RESULTS

Controls. Sixty lenses, which served as the controls for those eyes receiving 6, 10, 12, 14 and 18 Gy were studied. During the course of the study cataracts did not develop in any of the nonirradiated control lenses. When compared among themselves, specimens showed no changes by scanning electron microscopy. Fibers were readily distinguishable at the posterior pole

and were arranged in rows terminating at the suture line. Those specimens in which the fracture occurred spontaneously along the suture lines revealed a highly irregular, interdigitating surface where fibers from one side of the lens interface with fibers from the other side. Fibers in the nuclear region of the lens showed a highly-mottled, grape-like surface morphology. Larger structures such as ball-and-sockets appeared on the surface of the cortical fibers, and the cytoarchitecture was organized into tight hexagonal arrays. The more superficial fibers possessed fewer of the highly organized surface features seen in deeper fibers, and the fibers were arranged in parallel layers subcapsularly.

In the bow region, immature nucleated fiber cells consistently fractured through the cytoplasm, implying either a poorly organized intracellular structure or strongly adherent cells. The exposed nuclei of the bow region fiber cells were prolate structures, measuring $13\ \mu\text{m}$ by $1.6\ \mu\text{m}$ on average. Nuclear pores were seen and the average surface density was greater than 40 pores/ μm^2 . Nuclei were generally arranged in an orderly fashion, forming the characteristic bow pattern as seen in light microscopy and transmission electron microscopy. The bow curved anteriorly as one progressed toward the deeper fibers. In the anterior subcapsular region the epithelial cells were not readily distinguishable because the specimen fractured along intracellular lines with few intracellular organelles visible.

CATARACTOGENESIS AS A FUNCTION OF DOSE

1 + cataract. Clinical criteria for 1 + cataracts, as described by Merriam and Focht, are clusters of vacuoles and tiny opacities in the posterior superficial cortex and subcapsular region. Widening of the posterior suture lines is frequent.

Five rat lenses, irradiated with 6 Gy of x rays, were scored as stage 1 +. Debris of varying thickness, ranging from 2-30 μm , was seen along the capsule at the posterior pole and extended anteriorly for a short distance. The surface morphology of fibers just beneath this debris was smoother and the fibers themselves were edematous as compared to normal subcapsular fibers. The deeper fibers still retained their ultrastructural relationships. Occasional 10-40 μm oblate bodies, of which the surface morphology was similar to the more superficial fibers, were seen along the posterior capsule. Anteriorly, no change was seen in the epithelium, and the suture retained its rugged morphology.

Few discernible changes were seen in the bow region in comparison to

normal. The nuclei were less prolate having an average dimension of $12\mu\text{m} \times 2\mu\text{m}$ with a nuclear pore density of about $10 \text{ pores}/\mu\text{m}^2$. The nuclear dimensions of those lenses exposed to a dose of 10 Gy were comparable to that seen following 6 Gy, but the more superficial nuclei were positioned more posteriorly in the bow than in normal lenses.

2+ cataract. Four lenses which received a dose of 12 Gy developed opacities scored as 2+ on day 45 post-irradiation. A 2+ designation reflects moderately dense opacification within the posterior cortical region with frequent widening of the suture lines, in addition to some early opacification of the anterior superficial cortex.

The fiber interface along the more superficial posterior suture line was less rugged than that seen in 1+ cataract, and blebbing at the terminal ends was demonstrated. Subcapsular fibers were generally devoid of surface morphology, edematous, and showed progression into ellipsoidal globules with isthmus connections to their parent fibers. A grainy substance was noted within the superficial suture line. Fibers at the anterior pole were swollen and irregular though the surface morphology was unchanged. The epithelial cell layer showed no change. Few nuclei were seen in the bow region, but nonporous vesicles, approximately $4 \mu\text{m}$ in size, were found along the capsule. The structures are consistent with fragmented nuclei which occur following x-radiation insult. The lenticular nucleus remained unchanged.

3+ cataract. Stage 3+ cataract is defined as severe posterior cortical opacification with a loss of the red reflex. The anterior cortex shows extensive but not complete opacification.

Scanning electron micrographs of lenses exposed to 12 Gy demonstrated massive destruction and disorganization of the posterior cortex with the complete loss of organization along the suture line. Globules were present in the anterior cortical region just beneath the epithelium, as well as in the posterior subcapsular region. No changes were seen in the lenticular nucleus.

Two lenses observed 45 days after a 18 Gy dose of x rays demonstrated loss of the integrity of the cell fibers with occasional intracellular fracturing. The density of globules along the suture line and subcapsular region was greater in this case than that seen in 12 Gy cataracts. Similar pathological changes were seen along the anterior suture line but they were not as extensive as those seen at the posterior pole.

Marked disruption of the cytoarchitecture was observed in the bow/equatorial region. Fracture planes occurred, for the most part, extracellularly, in contrast to the intracellular splitting which was noted in early stage

cataracts and normal lenses. Cell surfaces were relatively smooth with globules seen among the fibers. The substance surrounding the fibers was grainier in comparison to earlier cataract stages and clear lenses. This grainy substance which extended along the posterior capsule toward the posterior pole had a texture similar to that seen in the suture lines. In a number of specimens, clefts were found in the equatorial cortex. These clefts were probably fluid-filled *in vivo*. Within them many globules of varying dimensions were seen. One such globule, unrestrained by adjacent fibers, formed in the cleft, and was still attached to its parent fiber by a 2 μm wide bridge.

The epithelium fractured along intracellular planes, yet no intracellular organelles or intracellular disruption were discernible. The lenticular nucleus remained unaltered compared to normal lenses.

4+ cataract. In a 4+ cataract, opacification of the anterior cortex is so severe that the posterior cortex cannot be seen. Eight lenses which reached a stage 4+ cataract were studied by scanning electron microscopy. Two received 12 Gy, the remainder received 18 Gy, yet no pathological difference was evident among these lenses. The changes in the posterior cortical region and bow/equatorial region were similar to that seen in 3+ cataracts, except that the grainy matrix was much more extensive. Damage to the anterior cortical region was more extensive as compared to 3+ and similar to the posterior cortex. In scanning across the lens substance from the central nucleus to the equator, minimal damage was seen in the nucleus with increasing disruption and destruction of the fibers going toward the equator. At the equator typical damage included fiber fragments, globules and a grainy matrix.

CATARACTOGENESIS AS A FUNCTION OF TIME

Slip-lamp biomicroscopy. Clinical examination of the lenses in the 3 to 4-week-old rats, both pre-irradiation and post-irradiation, showed no opacities. Extralenticular opacities were seen anteriorly on control and irradiated lenses and were probably remnants of the pupillary portion of the tunica vasculosa lentis. By week one opacities disappeared from the unirradiated eye, while capsular debris was seen in two of the irradiated lenses. The debris probably represents inflammatory elements normally found in conjunction with the retrogressing pupillary membrane, now perhaps increased in number due to the radiation insult. By the second week these opacities disappeared.

No lenticular opacities were seen until three weeks post-irradiation. At this stage 1-2 vacuoles were seen in most lenses in the posterior subcapsular re-

gion. By the fourth week most of the lenses reached a stage 1+ cataract (at least five vacuoles posteriorly). More subcapsular vacuoles were seen in both anterior and posterior poles. A few lenses showed changes along the suture lines and none demonstrated cortical changes. By the fifth week the opacities spread throughout the subcapsular area but were most dense at the poles.

All lenses had reached a stage 2+ cataract by the sixth week. The depth of the opacities at the poles increased, posterior pole more than anterior. Cataract progression over the subsequent weeks slowed markedly to where the cataracts reached stage 2.5+ by the twelfth week, and stages 3+ to 3.5+ by the 70th week.

One lens, which received a dose of 2 Gy, reached a stage 1+ cataract by the sixth week post-irradiation, yet never progressed beyond this stage during the year-long subsequent observation period.

Scanning electron microscopy. At 12 hours post-irradiation, blebbing of the anterior epithelium in the region of the germinative zone was found, and epithelial cells were found deep to fibers in the meridional row. By the second week two changes were seen in the bow region. The arc of the bow turned more posteriorly and the nuclei at the most posterior part of the bow were less prolate ($12\text{ }\mu\text{m} \times 2\text{ }\mu\text{m}$) than normal. The nuclear pore density was similar to normal lenses ($= 40\text{ pores}/\mu\text{m}^2$). Fibers immediately subjacent to the capsule were edematous without surface morphology. Occasionally ellipsoidal bodies, 10–40 μm in diameter, were seen subjacent to these fibers. By the third week the nuclei were shorter ($8\text{ }\mu\text{m} \times 2.5\text{ }\mu\text{m}$) and had fewer nuclear pores. A poor suture line was seen between fibers just subcapsularly at the posterior pole. Beneath these fibers, blebbing of the terminal ends of fibers at the suture was evident, followed by globule formation over the next few weeks. Over the subsequent months the globules disappear and a grainy matrix becomes prevalent along the capsule. By the 70th week post-irradiation, a shell of fibers without discernible defects had compressed the matrix into two thin layers of debris, one found subcapsularly and the other found midcortically.

When the lens which received a single dose of 2 Gy was examined by scanning electron microscopy, no discernible changes from normal lens morphology was observed in any region, especially subcapsularly.

DISCUSSION

Because of the refractile nature of the crystalline lens, a significant alteration of the architecture of the lens associated with ultrastructural changes

will be manifest as an opacity. In this study radiation-induced ultrastructural changes in the lens were noted, appeared in an orderly progression as a function of dose and time and accurately paralleled the gross clinical staging.

If the lens is exposed to a low dose and develops opacities, the surface of the more superficial, immature cell fibers become smoother than that of normal fibers, and the symmetry of arrangement of the fibers is slightly altered, more at the posterior pole than at the anterior pole. The nuclei of the cells in the bow region are shorter and wider. These changes can be a consequence of nuclear edema, fiber edema or both, with expansion of the nucleus due to the absence of external constraints. Debris ostensibly accreting from the degeneration of the immature, differentiating, superficial fibers is found in the region immediately beneath the posterior capsule.

Following higher doses, cataract development is progressive. So too are the severity of the cytopathological changes. Few nuclei are seen in the bow region, though 4 μm globules which might be nuclear fragments are noted. Superficial fibers appear more edematous and the interdigitations of the cell surfaces along the suture line are less obvious. Concomitant with the breakdown of these superficial fibers, 4-10 μm globules appear along the suture line. The globules are first observed in the posterior pole and appear later along the anterior suture line just deep to the epithelium. The appearance of such globules has been described in advanced stages of senile cataracts.²³

In later cataract stages, far more extensive changes are seen throughout the subcapsular and subepithelial regions. It is likely that degeneration of these fibers and globules contributes to the increased extracellular debris. Cortical fibers just deep to this region show edema and disruption, probably secondary to impaired transport of nutrients across the damaged superficial layer. The bow region is completely disrupted. At every stage the nuclear region of the lens appears unaffected.

It has been suggested that the site of radiation damage secondary to low linear energy transfer (LET) radiation, such as x rays, is the germinative zone within the anterior epithelium. The progeny of these germinal cells, which survive the insult, will continue to proliferate. It has been shown that in other mammalian cell lines *in vitro* one of the consequences of radiation insult is cellular transformation which is postulated to be secondary to chromosomal damage.^{24,25}

In the case of an irradiated lens, cataractogenesis is a consequence of active but altered fibrogenesis. The fibers produced are defective or potentially defective cells. The first cytological manifestation of the damage at the scan-

ning electron microscopic level is enlargement of the nuclei and a decrease in the nuclear pore density (pore density is inversely related to the initial radiation dose). Two weeks after the radiation insult, nuclei of cells that were in the germinative zone at the time of irradiation are found more posteriorly in the bow as compared to normal. Some of these cells will fail to differentiate fully into mature fibers, degenerate either anteriorly or posteriorly, or both and possibly die. Those cells which survive and fail to differentiate into mature fibers could be found along the posterior capsule as ellipsoidal masses. These cells have been described by Eshagian and Streeten as migrating cells.²⁶⁻²⁸ It is assumed that these migrating cells do not necessarily travel along the capsule, but are pushed along primarily toward the posterior pole between growing fibers. Fibers seen along the capsule within three weeks of the radiation insult were edematous without surface morphology and had blunted terminal ends. The cells may or may not mature into adult fibers, and the poor subcapsular suture line may be the consequence of these defective fibers which do reach the poles.

Those germinal cells which develop into mature fibers extending from the anterior to the posterior suture may manifest their defect by a different process. Globulogenesis has been defined as the breakdown of fibers at their terminal ends into membrane-bound globules.²³ During early globulogenesis, i.e., swelling of the terminal ends of the fibers at the suture, a thin layer of about 10 fibers between the capsule and the first swollen fiber ends is found. These fibers can be traced to that area just extracortically in the bow/equatorial region. It is in this region where nuclear degeneration occurs. Worgul et al.⁷ observed under the light microscope nuclear degeneration after fiber formation. It is suggested that those processes which lead to the breakdown of the nucleus would either render the fiber unstable or influence the inherently unstable fiber to breakdown. So at the time that nuclear degeneration is thought to occur, the terminal ends of the fibers show loss of the intricate surface morphology, followed by cytoarchitectural disruption and breakdown of the fibers. The extent of globulogenesis is a function of radiation dose. These fibers and globules disintegrate leaving liquefaction as seen in the grainy substance found in the suture lines and in the bow regions. At 10 Gy x rays, enough cells seemed to have survived the insult to continue to proliferate and differentiate normally and reconstitute a lens fiber cytoarchitecture which seems to be without defect. Most of the matrix is resorbed and all that remains is to thin layers of debris, one midcortically and the other subcapsularly.

SUMMARY

Cataracts produced in four-week-old rats following exposure of the eye to various doses of 185 kVp x rays were analyzed 45 days post-irradiation by scanning electron microscopy, as well as by conventional light microscopy. The severity and extent of the ultrastructural damage seen at scanning electron and light microscopic levels varied as an increasing function of dose and correlated well with the clinical changes which were scored by slit lamp biomicroscopy prior to preparation. In early stage low dose cataracts, damage was generally limited to the equatorial and posterior subcapsular regions. Late stage high dose cataracts were associated with clusters of 4-10 μm globules at the poles subcapsularly, massive disruption of the equatorial region and formation of a granular matrix that extended to the posterior pole. Ultrastructural changes were also noted in the superficial cortical region, while the lenticular nucleus and the remainder of the cortex remained unchanged.

In addition, cataract progression, as observed over a 70-week period in lenses irradiated with a single dose of 10 Gy x rays, was strongly time dependent. Fiber swelling seen in the bow region and posterior subcapsular area was followed by alterations in the suture immediately subjacent to the capsule. At five weeks fiber disruption at the suture was associated with the appearance of 4-10 μm membrane-bound globules. Over the subsequent weeks far more extensive damage was found, to the point where by the 70th week marked disruption of the architecture was seen throughout the entire lens.

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